Acute toxicity of polystyrene microplastics and cobalt assessed individually or in combination on "Amphibalanus amphitrite"

NOUSHEEN R.[1,2*], RITTSCHOF D.[2] and HASHMI I.[1]

[1] Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology, Sector H-12, Islamabad, Pakistan

[2] Duke University Marine Lab, Nicholas School of Environment, Duke University, USA

*corresponding author:

e-mail: rabiakhan172@gmail.com, rnousheen.phdiese@student.nust.edu.pk

Abstract Study was carried out to assess the ecotoxicity and interaction of polystyrene (PS) microspheres and trace element, cobalt (Co). Stage II nauplii of Amphibalanus amphitrite were exposed to environmentally relevant (250 and 1000 particles/ml) and future high (2000 particles/ml) concentrationss of 3µm PS microspheres in 3 different forms (unwashed, washed and aged). Series concentrations of Co were prepared, 24h LD50 was determined and 0.01, 0.1 and 1ppm Co concentrations were selected for use in individual and combined toxicity assays. It was found that microspheres of all types did not significantly affect the survival of nauplii (Mortality≤20%) at low concentrations. With increase in no. of particles in the medium (upto 2000 particles/ml), treatments consisting of unwashed and aged particles resulted in percentage mortality as high as 39% and 56% respectively however washed particles were still nontoxic. r. With regards to Co, mortality appeared to be a linear function of concentration.. When nauplii were exposed to a mixture of PS and Co, there was a shift in percentage mortality either towards low or high. Percentage mortality after combined exposure to Co at 0.01 ppm and unwashed/washed particles at 250 particles/ml was 13% and 37% greater than the mortality at same concentration of Co alone however with increase in no. of particles in the medium, a decrease in toxicity of cobalt was observed. Antagonistic effect of microplastics was more pronounced in the presence of aged particles and when present at 2000 particles/ml, toxicity of 1 ppm Co dropped down to half It is concluded that aging of microplastics, which is a real environment scenario, reduces the toxicity of cobalt even if present in higher concentrations in the environment.

Keywords: Polystyrene, Aged, Cobalt, Co-toxicity, Barnacle

1. Introduction

Plastics are widely used all over the world due to their versatile properties. However, inadequate disposal mechanisms and poor management of plastic waste is leading to its accumulation in the environment. Plastics ultimately end up in the oceans. According to an estimate, 150 million tones of plastic waste is present in the oceans (Jambeck et al., 2015) amongst which particles <5mm size, also known as microplastics, are the most abundant (Eriksen et al., 2014).

Microplastics can be ingested by the aquatic organisms and have the potential to move through the food chain. Filter feeding organisms are highly vulnerable to microplastics pollution due to their non selective feeding nature (Morphol et al., 2013). Microplastics have the ability to sorb different types of contaminants present in the aquatic environment thus it is important to study the way microplastics alter the bioavailability of sorbed contaminants (Sleight et al., 2017). Microplastics affinity towards other contaminants depends highly on its physical properties. Microplastics present in real environment rapidly get coated with biofilm (Vroom et al., 2017). Commercially available microplastics are supplied in the form of solutions and contain different additives. Different physical and chemical properties of microspheres, due to the presence of biofilm and additives, alter the way these tiny particles interact with other contaminants. Leachıng of unknown toxic chemicals used in production and process additives (such as antioxidant) from the virgin PS microspheres may also pose significant toxicity to marine biota (Martinez-Gomez et al., 2017). ,As microplastics used in the laboratory and those present in real environment differ in many aspects therefore to better understand the environmental consequences of such particles, a comparative insight into their toxicological potential is required which will also cover the gap that exists so far in microplastic's research. . Present study investigated the acute toxicity of polystyrene microplastics and cobalt , a trace element, both individually and in combination. Aim was to understand how different concentrations and types of microplastics change the toxicity and bioavailability of cobalt.

2. Materials and Methods

2.1. *Microspheres Washing and aging*

Red (3 µm) fluorescent microspheres (density: 1.05g/cm^3) were obtained from Duke Scientific Corporation in the form of an aqueous suspension packaged as 1% solids and contained surfactants to prevent aggregation and sodium azide to counter microbial growth. In order to remove preservatives, microspheres were washed using the protocol of Cole and Galloway (2015) with some modifications. From stock solutions, 10,050,000 red microspheres were separately added to 985 µl of HPLC grade water in a 1.5 ml polypropylene tube. Microspheres were centrifuged at 6800 rpm respectively for 5 minutes after which 800 µl of supernatant was withdrawn and replaced by the same volume of HPLC grade water. This washing process was repeated 4 times. Finally after removing 950µl of supernate, 950 µl gravity filtered sterile aged sea water (GFSASW) was added and mixed to get a secondary stock of red microspheres. Working solutions of 250, 1000 and 2000 particles per ml were prepared. Microspheres were aged according to Vroom *et al.* (2017). From the stock, 15 µl of red microspheres was added to tubes of 1.5ml capacity and washed using HPLC grade water as mentioned above. After washing the particles with GFSASW, aged sea water was added to half of the tubes to facilitate biofilm formation. Samples were mounted on wrist action shaker and mixed continuously for 2 months. After two months, mixing was stopped and aged samples were used in toxicity assays.

2.2 Preparation of cobalt solution

Stock solution (1000 ppm) of cobalt was prepared by dissolving 1 mg of cobalt chloride in 1 ml of Milli Q water (Karthikeyan et al., 2019). Working solutions of 0.01, 0.1, 1, 5, 10 and 15 ppm were prepared in aged sea water using aliquot from the stock.

2.3 *Acute toxicity assay*

Adult barnacles were scrapped from floating docks at Duke Marine lab during low tide and placed, soaked in aged seawater (ASW), in the dark room with a point light source at one end of the container to stimulate the release and enable collection of larvae. Larvae concentrated around light source were collected in 100 ml of aged filtered sea water and used within 3 hours of their release. Assay was conducted in 3ml glass tubes containing either 1ml of 22 micron gravity filtered ASW or exposure solutions. Stage II nauplii, 5-6 individuals, were transferred to each tube using glass Pasteur pipet and tubes maintained at 28 ºC and 12:12 L:D conditions for 1,2,3 and 24 h. Mortalities were recorded after stipulated time and specimens were preserved by addition of two drops of 30% formalin. Oven combusted glass ware was used and surfaces were properly cleaned with ethanol to avoid any contamination.

3. Results and Discussion

Present study was designed to investigate the toxicity of different concentrations and types of microplastics and their effect on toxicity/bioavailability of other contaminants like cobalt. Microplastics of all types were found to be non toxic (mortality ≤ 20%) at environmentally relevant concentrations (250, 500 and 1000 particles/ml). Polystyrene appeared to be significantly toxic to barnacle nauplii when they were exposed to 2000 particles/ml (Fig. 1). Percentage mortality at the highest concentration of unwashed and aged polystyrene microspheres was 40% and 60 % respectively. Increased mortality at the highest microplastic's concentration could be due to increased ingestion of microspheres. According to Caniff and Hoang (2018), nauplii are likely to ingest more microplastics if they encounter large number of particles in the medium. Toxicity of unwashed particles may be attributed to the presence of additives which not only affects directly but also indirectly by keeping the particles in suspension and increasing their bioavailability. Aging results in the formation of biofilm and alters the physical characteristics of microplastics. It is possible that aging produced microplastics that were brittle and their interaction with zooplankton resulted in enough damage thus leading to significant mortalities. It is also reported that zooplanktons prefer aged over pristine microplastics and therefore their increased uptake could be linked to the increased mortalities (Vroom et al., 2017).

Although cobalt is an essential trace element for many biological functions however it is significant to investigate the toxic effects of such metals as there are reports of their presence in coastal environment in high concentrations (Mansouri et al., 2015). Barnacle nauplii were exposed to different concentrations of cobalt for 24 hours and LD50 was calculated (Fig. 2). LD50 was found to be 4 ppm. Karthikeyan et al. (2019) also reported the 4ppm and 3.5 ppm as LC50 of cobalt for copepod and mysid shrimp after 96 h exposure which reveals that barnacle larvae is more sensitive to fluctuations in cobalt concentration.

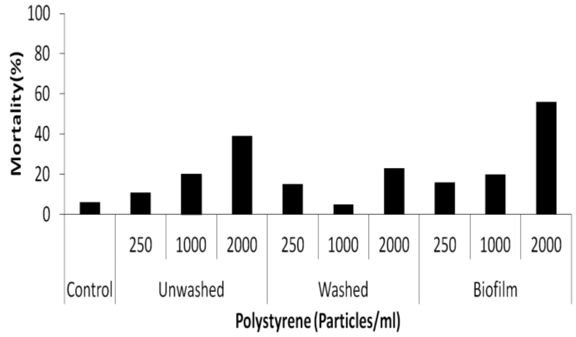

Figure 1. Percentage mortality of nauplii II after exposure to different concentrations and types of polystyrene microplastics

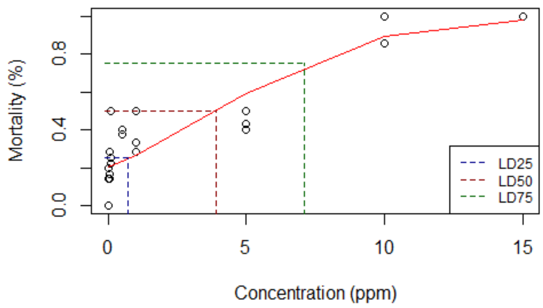

Figure 2. Acute toxicity of Cobalt to nauplii II and determination of LD50

Microplastics can interact with inorganic pollutants, including trace elements like cobalt (Co) and many others (Bradney *et al.*, 2019) therefore co-toxicity of microplastics and cobalt was assessed (Fig. 3) Microplastics considerably reduced the toxicity of cobalt and effect was pronounced when maximum concentration of cobalt and microplastics were used. Aged particles at 2000 particles/ml reduced the mortality due to 1ppm cobalt by 40% while unwashed and washed particles did not significantly alter the toxicity of cobalt. Presence of not significantly alter the toxicity of cobalt. Presence of biofilm fascilitated the sorption of metal which made the metal less bioavailable to barnacle larvae and resulted in significantly lower mortalties.

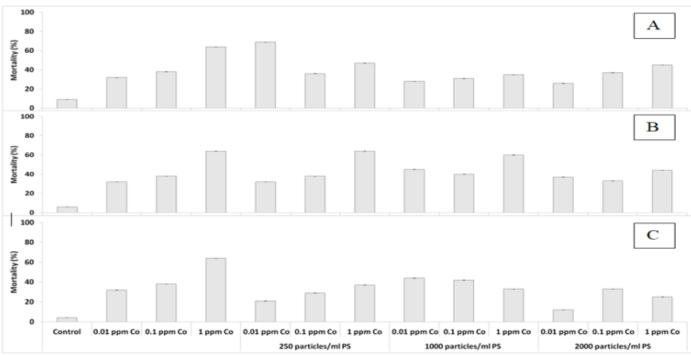

Figure 3. Percentage mortality of nauplii II after combined exposure to Cobalt and A) Unwashed B) Washed and C) Biofilm covered polystyrene microplastics.

4. Conclusion

This study shows that microplastics individually have no toxic effects. Contrastingly, cobalt showed significant toxicity and mortality of nauplii II was greater than 50% at 4 ppm concentration. Toxicity of cobalt considerably reduced when co-exposed with microplastics. Biofilm mainly controlled the toxicity of cobalt through sorption and resulted in increased survival of nauplii II even in cobalt rich environment. Findings suggest that microplastics remove the inorganic contaminants from the medium which positively affected the test organism in the study however on the other side it may result in food chain transfer of contaminants which needs to be explored.

, Palansooriya, K. N.,
lan, N. S., Ok, Y. S., ... &
). Particulate plastics as a
ment uptake by aquatic and
d human health
il, *131*, 104937.
ingestion by Daphnia
on algal growth. Sci Total Environ. 2018 Aug 15;633:500-507. doi: 10.1016/j.scitotenv.2018.03.176. Epub 2018 Mar 28. PMID: 29579661.

Eriksen, M., Lebreton, L. C., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., ... & Reisser, J. (2014). Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PloS one*, *9*(12), e111913.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., ... & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768-771.

Karthikeyan, P., Marigoudar, S. R., Nagarjuna, A., & Sharma, K. V. (2019). Toxicity assessment of cobalt and selenium on marine diatoms and copepods. *Environmental Chemistry and Ecotoxicology*, *1*, 36-42.

Mansouri, B., Maleki, A., Johari, S. A., & Reshahmanish, N. (2015). Effects of cobalt oxide nanoparticles and cobalt ions on gill histopathology of zebrafish (Danio rerio). *Aquaculture, Aquarium, Conservation & Legislation*, *8*(3), 438-444.

Martínez-Gómez, C., León, V. M., Calles, S., Gomáriz-Olcina, M., & Vethaak, A. D. (2017). The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. *Mar. Environ. Res*, *130*, 69-76.

Paig-Tran, E. M., Kleinteich, T., & Summers, A. P. (2013). The filter pads and filtration mechanisms of the devil rays: variation at macro and microscopic scales. *Journal of Morphology*, *274*(9), 1026-1043.

Sleight, V. A., Bakir, A., Thompson, R. C., & Henry, T. B. (2017). Assessment of microplastic-sorbed contaminant bioavailability through analysis of biomarker gene expression in larval zebrafish. *Marine pollution bulletin*, *116*(1-2), 291-297.